Physicochemical Analysis of Gelatin Extracted from African Catfish Skins using *Nephelium lappaceum* (Rambutan) Vinegar

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DECLARATION

I hereby declare that the work embodied in this report is based on my original research except for citations and quotations which have been duly acknowledged.

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%	percent
±	plus-minus sign
≤	less than or equal to
°C	degree celcius
*a**	redness
*b**	yellowness
cm	centimeter
g	gram
kg	kilogram
*L**	lightness
Mj	miliJoule
ml	milimeter
mm/s	milimeter / second
n	number of replication
w/v	weight/volume
pH	potential of hydrogen
N	Newton

LIST OF ABBREVIATIONS

Reference No.

BSE	Bovine Spongiform Encepthalopathy
FMD	Foot and Mouth Disease
GME	Gelatin Manufacturers of Europe
GMIA	Gelatin Manufacture Institute of America
HCl	hydrochloric acid
CH_3COOH	acetic acid
H_2SO_4	sulphuric acid
NaCl	sodium chloride
NaOH	Sodium hydroxide

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ABSTRACT

Gelatin is a biopolymer with broad range of applications in food, pharmaceutical and photography industries. The main sources of gelatin are from the skins and bones of porcine and bovine. Due to religious sentiments by Muslim and Hindu communities as well as viral diseases such as bovine spongiform encephalopathy (BSE), an alternative was identified to replace mammal-derived gelatin which is from fish sources. In this study, African catfish was chosen for extraction of gelatin due to high content of collagen in its skin. Rambutan vinegar is used as pre-treatment acid in the study in order to identify whether natural vinegar could be a possible alternative to replace the use of synthetic acetic acid in gelatin pre-treatment process. Thus, the objective of this study is to optimize the gelatin extraction from catfish skin pre-treated with Rambutan vinegar and acetic acid of varying concentrations (2%, 4% and 6%). The physicochemical properties of gelatin extracted were evaluated in terms of yield, pH, colour, texture, moisture, ash and protein analysis. The highest yield is obtained from 6% acetic acid which is 17.36%. The pH of the gelatin pre-treated with acetic acid ranged from 4.67 to 4.91 whereas, the gelatin pre-treated with Rambutan vinegar ranged from 5.02 to 5.71. There were significant ($p \leq 0.05$) different in colour and texture of gelatin between all the gelatin treatments. All gelatin pre-treated with Rambutan vinegar gave positive results where low moisture (7.80-8.91%), low ash (0.77-0.82%) and high protein content (89.70-95.77%) were obtained compared with gelatin pre-treated with acetic acid. The optimum concentration of Rambutan vinegar that is suitable for gelatin acid pre-treatments was 6% as it obtained the highest yield and better physicochemical properties compared with 2% and 4%. Therefore, Rambutan vinegar can be an alternative to replace acetic acid as gelatin pre-treatments acid.

Keywords: gelatin extraction, catfish skin, Rambutan vinegar, acetic acid, physicochemical analysis.

Analisis Fizikokimia Gelatin Diekstrak daripada Kulit Ikan Keli Afrika

Menggunakan Cuka Rambutan (*Nephelium Lappaceum*)

ABSTRAK

Gelatin ialah biopolimer yang mempunyai pelbagai aplikasi dalam industri makanan, farmaseutikal dan fotografi. Sumber utama gelatin adalah daripada kulit dan tulang khinzir dan lembu. Disebabkan oleh sentimen agama oleh komuniti Islam dan Hindu, serta penyakit virus seperti 'bovine spongiform encephalopathy' (BSE), satu alternative telah dikenalpasti itu menggantikan gelatin yang berasal dari mamalia, iaitu daripada sumber ikan. Dalam kajian ini, ikan keli Afrika dipilih untuk pengekstrakan gelatin disebabkan oleh kandungan kolagennya yang tinggi. Cuka rambutan digunakan sebagai asid pra-rawatan dalam kajian ini untuk mengenal pasti sama ada cuka semulajadi boleh menjadi alternatif untuk menggantikan penggunaan asid asetik sintetik dalam proses pra-rawatan gelatin Oleh itu, objektif kajian ini ialah mengoptimumkan pengekstrakan gelatin dari kulit ikan keli yang telah dipra-rawat dengan cuka rambutan dan asid asetik yang berbeza kepekatan (2%, 4% dan 6%). Sifat fizikokimia gelatin yang diekstrak dinilai dari segi hasil, pH, warna, tekstur, kelembapan, abu dan protein. Hasil tertinggi diperolehi daripada 6% asid asetik iaitu 17.36%. pH gelatin yang telah dirawat dengan asid asetik adalah dalam lingkungan 4.67 hingga 4.91, manakala gelatin yang telah dirawat dengan cuka rambutan adalah dalam lingkungan 5.02 hingga 5.71. Terdapat perbezaan yang signifikan ($p \leq 0.05$) antara semua rawatan daripada segi warna dan tekstur gelatin. Semua gelatin yang dipra-rawat dengan cuka rambutan memberi keputusan positif di mana ia memperoleh kandungan kelembapan (7.80-8.91%) dan abu (0.77-0.82%) yang rendah serta kandungan protein (89.70-95.77%) yang tinggi berbanding gelatin yang dipra-rawat dengan asid asetik. Kepekatan optimum cuka rambutan yang sesuai untuk pra-rawatan gelatin ialah 6% kerana ia memperolehi hasil tertinggi and ciri fizikokimia yang lebih baik berbanding 2% and 4%. Oleh itu, cuka rambutan boleh menjadi alternatif untuk menggantikan asid asetik sebagai asid pra-rawatan untuk gelatin.

Kata kunci: pengekstrakan gelatin, kulit ikan keli, cuka rambutan, asid asetik, analisis fizikokimia.

CHAPTER 1

INTRODUCTION

1.1 Research background

Gelatin is a fibrous protein obtained through partial hydrolysis of collagen, which is commonly extracted from connective tissues, bones, and skins of animals. It is a colourless, brittle, translucent and flavourless solid substance (Irwandi et al., 2009). The demand for gelatin has been increasing from years to years. According to report by Gelatin Manufacturers of Europe [GME] (2009), the annual world production of gelatin is almost 326,000 tons, where the highest production is from bovine skin with 44%, bovine hides (28%), bones (27%), and other sources (1%). The other sources stated include gelatin production from poultry and fish sources.

There is a broad range of gelatin applications in food, medical, pharmaceutical and photographic industries (Karim & Bhat, 2009; Hao et al, 2009). Gimenez, Gormez-Guillen and Montero (2005a) said that gelatin functions in enhancing the consistency, elasticity and stability of food products. Gelatin quality is important as it influence the functional properties of gelatin such as formation of gel, water absorption capacity and

stabilize emulsion. Previous study by Gomez-Guillen, Turnay, Fernandez-Diaz, Ulmo, Lizarbe, & Montero (2002) showed that factors that affects the gelatin quality are the types of tissue, animal species, physicochemical properties, and manufacturing methods.

However, there are alarming issues where porcine gelatin are rejected by Muslim communities as they are prohibited from consuming porcine and its by-products. Besides, bovine spongiform encephalopathy (BSE) and food-and-mouth (FMD) occurrences have increased the efforts to find another gelatin sources. Gelatin extraction from poultry source is less concern because of the risk of avian influenza transmission (Saito, Kiyose, Higuchi, Uchida & Suzuki, 2009). Therefore, by-products waste of fish which is in the forms of skins and bones can be introduced as an alternative for gelatin extraction (Gomez-Guillen et al., 2002).

The gelatin extracted from freshwater fish such as catfish, tilapia and snakehead give higher yield compared to other fish (Songchotikunpan, Tattiyakul & Supaphol, 2008). In 2007, catfish production in Malaysia gained RM107 million from RM481 million from the total aquaculture fish production (Department of Fisheries Malaysia, 2007). The gels from catfish skins show good gelling ability and thermally non-degradable (Gomez-Guillen et al., 2002). Thus, catfish skin can become an alternative raw material for gelatin production.

Mokhtar et al. (2016) stated that fruit-based vinegar such as Rambutan vinegar, Dokong vinegar and Nipah vinegar have the capability to compete with artificial vinegar products in the market due to its health benefits as well as being wholly made from pure fruit juice. Furthermore, the raw material for the production of natural vinegar is cheap and readily available. Nonetheless, natural vinegar production has not been commercialized in Malaysia and only been manufactured in small-scale industry. The

concentration of acetic acid in natural vinegar commonly ranges between 4-8% and might reach up to 18% for picklings. So, there is possibility for natural vinegar to become an alternative to synthetic acetic acid in the gelatin extraction.

Therefore, this study focused on extraction of gelatin from African catfish skin using Rambutan vinegar with different concentration values to obtain high yield of gelatin. Also, the study aim in producing the gelatin extracted using Rambutan vinegar that has a comparable physicochemical characteristics with gelatin extracted using synthetic acetic acid by conducting pH, colour, texture, moisture, ash and protein analysis.

1.2 Problem Statement

Generally, the commercial gelatin in the market is made up from skin and bones of bovine and porcine. This issue creates concern among consumers with regard to its usage. This is mainly due to religious beliefs where Islam forbid the consumption of any pork-related products, while Hindus do not consume cow-related products (Karim & Bhat, 2009).

Therefore, alternative has been taken by producing gelatin from other sources like poultry and fish. The extraction of gelatin from poultry slaughtered wastes has also been studied, but with less emphasis due to the risk of avian influenza transmission (Saito et al., 2009). Commercial production of gelatin made from poultry skins and bones is limited due to low yields. Moreover, poultry skins are commonly used as raw material for other food applications (Karim & Bhat, 2009; Schrieber & Gareis, 2007).

Meanwhile, fish processing industries specifically filleting industry is becoming popular in Malaysia. The by-product wastes from the fish filleting process can reach up until 75% of the total catch weight (Shahidi, Xiao-Qing & Synowiecki, 1995). The wastes which consist of fish skins and bones have high content of collagen which can be alternative sources of gelatin. (Gomez-Guillen et al., 2002). Freshwater fish like catfish has high content of collagen in its skin. Thus, it is possible for catfish to become new material for gelatin production.

In this study, the use of common synthetic acetic acid during pre-treatment was replaced with natural vinegar which is Rambutan vinegar. Currently, there is no study regarding the use of Rambutan vinegar as pre-treatment medium in gelatin extraction. Synthetic acetic acid is more favourable compared to acetic acid from natural vinegar. This is due to Rambutan vinegar has a higher price and long fermentation process of six to eight weeks compared to synthetic acetic acid (Mokhtar et al, 2016).

Despite of that, natural vinegar is preferable by the consumers who are concern about their health by choosing a healthier choice of diet even if it is higher in cost. Natural vinegar gives a lot of benefits to internal body organs mainly to intestines. Thus, this research aims in conducting the gelatin extraction by using varying concentration values of Rambutan vinegar which are 2%, 4% and 6%. Then, the yields and physicochemical properties of the gelatin extracted with Rambutan vinegar was analysed and compared with gelatin extracted with synthetic acetic acid.

1.3 Hypothesis

H_0: There is no significant difference in the gelatin extracted from African catfish skin using Rambutan vinegar of different concentrations in terms of yield of extraction and physicochemical quality of gelatin.

H_A: There is a significant difference in the gelatin extracted from African catfish skin using Rambutan vinegar of different concentrations in terms of yield of extraction and physicochemical quality of gelatin.

1.4 Objectives

1.4.1 To optimize the gelatin extraction from African catfish skin pre-treated with different concentrations of Rambutan vinegar.

1.4.2 To compare the physicochemical properties of gelatin extracted from African catfish skin in terms of pH, colour, texture, moisture, ash and protein analysis.

1.5 Scope of Study

This study focused on gelatin extracted from warm water fish spesies specifically, African catfish skins. Also, the pre-treatments process for this study was conducted by

only using Rambutan vinegar as pre-treatments acid instead of other natural vinegars, alongside with acetic acid. Other than that, the physicochemical properties of the African catfish skin gelatin were analysed in terms of yield, pH, colour, texture, moisture, ash and protein analysis. Then, the physicochemical properties of the gelatin were compared with gelatin pre-treated with acetic acid to determine the best quality of gelatin extracted.

1.6 Significance of Study

There are numerous studies on gelatin extraction from mammals specifically bovine and porcine. However, only a few researches have been done on freshwater fish gelatin. This study is aimed to provide more insight towards warm water fish gelatin as an alternative to mammalian gelatins, with more emphasis on methods of extraction and its physicochemical properties. These warm water fish species give greater functional properties of gelatin compared to cold water fish species (Gilsenan & Ross-Murphy, 2000).

In addition to that, there is no study yet found on the use of Rambutan vinegar as pre-treatment for the extraction of gelatin. Therefore, the aim is to optimize the gelatin extraction using Rambutan vinegar of different concentration values. There is a possibility for Rambutan vinegar as an alternative to replace synthetic vinegar in the gelatin extraction if it obtained high yield and has a better physicochemical properties. Thus, commercialization of Rambutan vinegar as medium for pre-treatment in gelatin extraction can be done.

CHAPTER 2

LITERATURE REVIEW

2.1 Gelatin

According to Belitz, Grosch and Schieberle (2004), gelatin is a pure protein, produces by partial thermal hydrolysis of collagen; a major protein constituent of animal connective tissues. The hydrolysis caused a destruction of cross-linkages between collagen polypeptide chains alongside with few breakage of polypeptide bonds. Hence, a treatment is required in order to convert collagen into gelatin that will break the non-covalent bonds which caused adequate swelling and cleavage of intra- and intermolecular bonds, hence, leads to collagen solubilization (Stainsby 1987).

In 2009, Karim and Bhat states that 46% of gelatin is made from porcine skins, followed by bovine skins (29.4%), bones (23.1%) and other sources (1.5%) (See, Hong, Ng, Wan Aida & Babji, 2010; Lim, Oh & Kim, 2001). Gelatin is a translucent solid substance which is colourless or slightly yellow, brittle, odourless and tasteless (Sakr, 1997). Nowadays, gelatin is available in in the form of powders, sheets and leaves. Gelatin is the only hydrocolloid which is not a carbohydrate that is widely used in food, pharmaceuticals and cosmetics industries (Nelson & Cox, 2005).

2.1.1 Properties of Gelatin

The physical properties of gelatin include characteristics such as colourless or slightly yellow, transparent, brittle and tasteless (Sakr, 1997). Gelatin is soluble in hot water, acetic acid and glycerol but insoluble in organic solvents. Gelatin has a unique properties that enable it to form thermo-reversible gel (Jamilah & Havinder, 2002; Zhou & Regenstein, 2005) at melting temperature close to body temperature with good solubility in water (Norziah, Al-Hassan, Khairulnizam, Mordi, & Norita, 2009). Nevertheless, gelatin can melt at low temperatures around 35°C for beef gelatin and 15°C for fish gelatin (Haug, Draget, & Smidsrod, 2004). Consequently, gelatin gel is not ideal for recipes involving heating at high temperature as the gel would become liquid and collapse in a short time.

Gelatin has specific structure as it consists of 20 amino acids thus, giving it a broad range of functionality (Norziah et al., 2008). Gelatin properties are influenced by species of animals, age and type of collagen tissues. Physicochemical properties and the variation of production methods significantly influenced the gelatin quality (Gomez-Guillen et al., 2002). According to Gimenez, Turnay, Lizarbe, Montero & Gomez-Guillen (2005b), food grade gelatin depends on rheological properties, colour, translucence, flavour and solubility.

Gelatin is obtained from collagen, hence, the properties of the extracted gelatin is influenced by the type and source of collagen. The molecular structure of gelatin is mainly a multiple repetitions of a Gly-X-Y sequence, which is similarly to collagen. Gly stands for glycine, X is proline (Pro) whereas Y is hydroxyproline (Hyp) (Ergel & Bachinger

2005). Karim and Bhat (2009) states that collagen in vertebrates familiarly contain around 35% glycine, 21% proline, 11% alanine and hydroxyproline. Molecules in collagen are stabilized by some intermolecular bonds and hydrogen bonds which are composed of three helical polypeptide chains. Each chain contain 1000 amino acids and become intertwined to form a stable triple helix that may vary in size.

Collagen can be categorised into few types. The most common is Type I collagen, mainly in connective tissues, skins, tendons and bones. Type II collagen usually present in cartilage tissues whereas Type III collagen is highly influenced by the age. A young skin can contain up to 50% of collagen, but as time increase, the collagen decreased up to 5-10%. Other collagen types are present in small quantities, primarily in organs such as membranes, cornea, heart muscle, lungs as well as intestinal mucosa (Schrieber & Gareis, 2007).

2.1.2 Applications of Gelatin

Gelatin is an important biopolymer that is widely used in food, cosmetic, pharmaceutical and photographic industries (Karim & Bhat, 2009). Gelatin has a good biodegradability and biocompatibility in physiological environments as well as low in cost. Because of that reason, its function is expanding to new application as functional foods.

In the food industry, gelatin is used extensively in products like candies, desserts, dairy products, jelled meats, ice cream and some bakery products. Gelatin has a great demand in food industry due to its functional properties such as water absorption capacity,

formation of gel as well as the ability to stabilize emulsions. In addition to that, gelatin can act as ingredient to enhance stability, elasticity and consistency of food products (Gimenez, et al. 2005a).

Other than that, gelatin can function as thickener agent, emulsifier, and binder, thus, replacing the function of fat. Besides, gelatin has a comparable sensory quality with fat and gives no negative impact on the taste of food products. Gelatin is usually used as a gelling and foaming agent in some culinary preparations. Gels made with gelatin are appreciated by cooks and consumers due to its distinct texture feature which is soft and melts easily in mouth before swallowing (Baziwane & He, 2003).

Gelatin is used as a stabilizer in dairy products and fermentation as it provides chewiness, texture and foam stabilization (Gimenez et al., 2005a). This includes in ice cream production as gelatin give a fine, smooth and flexible gel texture to the products. Besides, gelatin is broadly used as a dietetic agent in management of obesity due to its low calorie content and in baby food (Riaz & Chaudry, 2004). Gelatin has high protein content which can enhance protein levels and applicable in body-building foods.

In the pharmaceutical fields, gelatin is used in production of medication capsule gel; including soft and hard capsules (Karim & Bhat, 2009), plasma expanders, adsorbent pads and wound dressing (Demirhan, Ulca & Senyuva, 2012). Gelatin is utilized as matrix implants, in intravenous infusions and in injectable drug delivery microspheres. There are also reports in which vaccines used for immunization against measles and rubella contain gelatin as a stabilizer

Gelatin is also applied as a vehicle for drugs, proteins and genes, as well as a substitute for human skin, blood vessels and ligaments (Gomez-Guillen, Gimenez, Lopez-Caballer & Montero, 2011). Gelatin is also applied in edible film production, make

it suitable for applications in photographic applications (Jongjareonrak, Benjakul, Visessanguan & Tanaka, 2006).

2.2 Source of Gelatin

Most of gelatin marketed worldwide are made from porcine and bovine sources. Gelatin is extracted from animal connective tissues, bones and skins. Issues of halal food for Muslims also the spread of mad cow disease (BSE) increased the efforts to find another sources of gelatin.

The global demand for gelatin has been increasing annually. Recent reports indicate that the annual world production of gelatin is nearly 326,000 tons, with pig skin derived gelatin accounting for the highest (44%) output, followed by bovine hides (28%), bones (27%), and 1% from other sources (GME, 2009). By-products of poultry and fish are rarely used as a material for the production of gelatin. The extraction of gelatin from poultry slaughtered wastes has also been studied but with less corcern (Saito et al., 2009).

Fish processing industries specifically filleting industry is getting popular. Fish filleting process can produce by-product wastes that may reach up to 75% of the total catch weight (Shahidi et al., 1995). The waste which consisting of fish skins and bones are have high content of collagen which can be a potential source of gelatin to replace mammalian gelatin.

2.2.1 Issue on Gelatin

Most of commercial gelatin is currently extracted from bovine and porcine sources. Karim & Bhat (2009) stated that 41% of the gelatin produced worldwide is sourced from porcine skins followed by 29.5% from bovine bones. However, the gelatin produced by those two sources cannot be used widely all over the world due to religious prohibitions (Sadowska, Kolodziejska & Niecikowska, 2003). Muslim do not accept any porcine related food products. Besides, bovine gelatin is acceptable only if it has been processed according to their religious requirements.

Furthermore, people are concerned about the outbreaks of bovine spongiform encephalopathy (BSE) disease and food-and-mouth disease (FMD). BSE are caused by the accumulation of the pathological prion protein in the brain and central nervous system, which affects adult bovines (Toldra et al., 2012). Karim and Bhat (2009) state that there is also limited use of bovine gelatin as Hindu do not accept products that related to bovine for consumption. In addition, there is a strong competition exists regarding the procurement of porcine or other mammalian sources among manufacturers which lead to increased demand and raised costs of gelatin from the sources. Therefore, gelatin derived from poultry and fish has obtained an intense interest in the market.

There are some researches on gelatin extraction from poultry slaughter wastes, but with less emphasis due to the risk of avian influenza transmission (Saito et al., 2009). Commercial production of gelatin made from poultry skins and bones is limited due to its low yields. Also, poultry skins are used as raw material for food applications (Schrieber & Gareis, 2007).

For that reason, gelatin made up from fish by-products has the possibility to become an alternative which has higher acceptability compared to bovine gelatin from the aspects of religious and food safety issues. The processing of fish by-products can convert a low value product or product that requires high cost in its disposal into a product that is able to cover all the costs of processing, production and disposal with consequent higher added value and decreased environmental harms (Schrieber & Gareis, 2007).

2.2.2 Gelatin from Fish Source

Fish gelatin are gained from the fish skins and bones. The wastes from fish processing in food industry can reach as much as 75% of the total catch weight (Shahidi, 1995). 30% of such waste are skin and bones provided with high collagen content which could be used to make gelatin (Gomez-Guillen et al., 2002). According to Arnesen and Gildberg (2006), the production of fish gelatin only contribute to only about 1% of the annual production of gelatin worldwide.

Fish gelatin is a better alternative to replace mammalian gelatins as it is acceptable by Muslim communities and free from serious health risks such as bovine spongiform encephalopathy (BSE) and foot-and-mouth disease (FMD). Norziah et al. (2008) mentioned that fish gelatin is different from other sources of gelatin in the physicochemical properties such as melting and gelling temperatures as well as the strength of gel. The difference of the properties is due to the different compositions of amino acid specifically proline and hydroxyproline contents inside the gelatin (Haug et al., 2004).

Previous studies have proven that warm water fish contains higher amino acids content than cold water fish spesies (Gudmundsson & Hafsteinsson, 1997). Due to that, the warm water fish produced more gelatin yields compared to cold water fish. Nonetheless, it is depends by their species and processing method. Thus, Malaysia as a tropical country and produce warm water fish species should take this opportunity to manufacture gelatin from warm water fish.

According to Department of Fisheries Malaysia (2007), freshwater aquaculture contributed 29.1% of the total aquaculture production and this value increase by 12% which is from 61 652.48 tons to 70 064.27 tons in 2007. The main freshwater species of fish cultured were red tilapia (26,175.33 tons), catfish (21,891.55 tons), black tilapia (5,848.98 tons) and Pangasius catfish (5,784.44 tons).

Malaysia's fish based industry such as fillet processing and surimi industries are developing progressively as there is increasing in demands of fish-based products in the market (See et al., 2010). These industries only utilized the fish flesh and discard the skins, fins and bones. See et al. (2010) also mentioned that freshwater fish skins which comprising around 5% of the whole fish, and surely can become source of raw material for gelatin production. This action can contribute to reduction of fish by-products waste and produces a value-added product that would help to strengthen the economy of the industry.

Fish gelatin must have the following criteria in order for it to be applied in food and pharmaceutical industries. Firstly, the fish by-products must be in high quantity and its economical collection are essential to be constanty produced in the industry. Next, gelatin from fish by-products must have a good rheological properties such as gelling and melting points as well as gel strength at comparable level with mammalian gelatin (Cho,

Gu & Kim, 2005). Therefore, fish gelatin has been highlighted as an alternative to mammalian gelatin for its particular qualities such as a low melting point which results in dissolve faster in the mouth with no remaining 'chewy' mouth feel. In addition to that, physicochemical properties such as colour, odour, taste, solubility, transparency of gelatin are also important (Gomez-Guillen et al., 2011).

There is very limited information of collagen derived from fresh water fish as an alternative gelatin source. Only few studies have been conducted on warm water fish gelatin and these studies showed that these species give a better functional properties of gelatin rather than cold water fish species (Gilsenan & Ross-Murphy, 2000). Even though gelatin from warm-water fish species might have similar physicochemical properties to that gelatin from mammalian sources, researchers have specified that it depends on the fish species, type of raw material and processing conditions (Muyonga, Cole, & Duodu, 2004).

2.2.3 Gelatin from African Catfish Skin

Figure 2.1: African catfish or its scientific name, *Clarias gariepinus.*

Clarias gariepinus or commonly known as African catfish is warm water fish which is commonly farm-raised and supplies large quantity of fish skins annually. The gels from catfish skins show good gelling ability and thermally non-degradable (Gomez-Guillen et al. 2002). Thus, African catfish skin can become an alternative raw material for gelatin production. Table 2.1 shows the scientific classification of African catfish:

Table 2.1: Scientific classification of African catfish.

Kingdom	**Animalia**
Phylum	Chordata
Class	Actinopterygii
Order	Siluriformes
Family	Clariidae
Genus	Clarias
Species	*C. gariepinus*

A study by Songchotikunpan et al. (2008) discovered that the yield of gelatins extracted from freshwater fish such as catfish, snakehead, red tilapia and Pangasius catfish were higher compared to the gelatins extracted from other fish. The variation is influenced by the differences in skins proximate composition, the content of collagen and the quantity of soluble components in the skins.

Moreover, these properties vary with the fish species, age and the extraction methods used. According to Department of Fisheries Malaysia (2007), the total amount of catfish production in 2007 was 21891.55 metric tons. In that year, the catfish production obtained RM107 million from RM481 million of the total aquaculture fish production in Malaysia. Until now, gelatin from the skins of African catfish has not been systematically studied as a raw material for edible gelatin.

2.3 Gelatin Extraction Method

Gelatin is a protein compound that derived from denatured collagen. A pre-treatment process is needed in order to obtain gelatin by converting the collagen tissues into a substance which is suitable for extraction (Gimenez et al., 2005a), the fish can be extracted using two methods; an acid and alkaline processes.

During fish gelatin extraction, acid process is the extraction procedure that is conducted in an acid solution (Gomez-Guillen et al., 2002). In most cases, acid pre-treatment is applied before conducting the gelatin extraction. Meanwhile, the alkaline process is pre-treatment of fish skin with an alkaline medium, which is commonly followed by neutralization with an acid solution. Therefore, it can be concluded that the extraction can be carried out whether in an alkaline, acid, or neutral processes. (Jamilah & Harvinder, 2002; Gomez-Guillen et al., 2002).

Diluted acetic acid produces a better gelling gelatins compared to citric acid (Gomez-Guillen et al., 2002). Ahmad and Benjakul (2011) mentioned that extraction process need to be optimized in order to obtain high gelatin yields with desired properties

and functionalities. Gelatin extraction is conducted after an appropriate acid pre-treatment with proper extraction time. Acetic acid is the common solvent used in collagen preparation as high extractability and the solubility of collagen plays an important role in the extraction.

2.3.1 Pre-treatment Methods

Acid pre-treatments for gelatin extraction commonly used hydrochloric acid (HCl), sulphuric acid (H_2SO_4) and phosphoric acid (H_3PO_4) to extract gelatin from young collagen that have no complex structure such as porcine skin and several fish spesies within 10-48 hours (Rahman & Jamalulail, 2012; Hinterwaldner, 1977). The gelatin obtained is known as type A gelatin. Acid pretreatment removes some fat, impurities and acid soluble proteins, then hot water extraction causes collagen hydrolysis which then transform it into gelatin

Meanwhile, alkali pre-treatments used sodium hydroxide (NaOH) or potassium hydroxide (KOH) for extracting gelatin from matured collagen which have complex cross structure such as skins and bones of cattles, which takes a longer period of time from 6-20 days in order to dissolve the complex structure of collagen (Riaz & Chaudry, 2004). This gelatin is called as type B gelatin. Alkali pretreatments aid in removal of non-collagenous protein without collagen loss. The method also destroy few cross-linked chemicals that were still presence in the collagen and to remove unwanted materials

There are other food acids can be used such as citric acid ($C_6H_8O_7$) or lactic acid ($C_3H_6O_3$). Also, variety of fish species may be used for gelatin extraction. Alkali

hydrolysis can increase the bloom strength and speed the production process. However, alkali is rarely used in the manufacturing of most fish-based gelatins.

The yield and quality of gelatin are affected by the species of animals, type of tissues, and extraction process; which may depend on pH, concentration, temperature, and time during both pre-treatment and extraction (Cho, Jahncke, Chin & Eun, 2006; Zhou & Regenstein, 2004; Montero & Gomez- Guillen, 2000). Therefore, optimization of extraction method used will enhance the extraction of African catfish skin gelatin. The combination of acid and alkali pre-treatments gives no significant collagen loss and maintains the structural integrity which represents higher convenience in terms of applications in industry.

2.3.2 Natural Vinegar

Vinegar can be defined as any liquid fit for human consumption which is produced from a suitable raw material of agricultural origin, that contained either starch or sugars by double fermentation process (Mokhtar,et al., 2016; Food and Agriculture Organization/World Health Organization [FAO/WHO], 1987). Fruit vinegars were formed from the fermentation of fruit juices. Vinegar is produced from ethanol fermentation that yields the main ingredient which is acetic acid (CH_3COOH). It is a product of a mixed fermentation of yeast proceeded by acetic acid bacteria

The acetic acid concentration normally ranges from 4-8% by volume for table vinegar and can reach up to 18% for pickling. According to Malaysian Food Regulation (1985), vinegar is a liquid product prepared from the alcoholic fermentation or

fermentation of any suitable food. In addition, the vinegar should contain more than 4% w/v of acetic acid and should not contain any mineral acid.

Natural vinegars usually contain small amounts of citric acid ($C_6H_8O_7$), tartaric acid ($C_4H_6O_6$) and other acids. Vinegar is mainly used to flavour and preserve food and also become one of the ingredient in salad dressings. Othaman, Sharifudin, Mansor, Kahar and Long (2014) stated that natural vinegar is a better food additive compared to synthetic vinegar as it carries important amino acids from its fruit source and can act as a medicine for gastric troubles. The beneficial effect of acetic acid in vinegar is it altered the metabolic processes in the liver and digestive tract (Johnston & Gaas, 2006).

However, natural vinegar production is unfavourable among the manufacturers due to long fermentation period that can extend up to 6-8 weeks and the substrates availability. (Mokhtar et al., 2016). In addition, the cost of natural vinegar is higher than synthetic vinegar in local market. The production of natural vinegar is only been practised in small-scale industry and has not been commercialized in Malaysia (Karim, Ismail, Daud, & Alam, 2011) by using substrates from various types of agro-based products such as nipah sap, coconut sap, and matured fruit juice.

Therefore, this study will determine the possibility of natural vinegar, specifically Rambutan vinegar to replace the use of synthetic acetic acid in gelatin extraction, thus, increase the functionality of Rambutan vinegar.

2.3.3 Pre-treatment of gelatin using Rambutan vinegar

Rambutan or its scientific name; *Nephelium lappaceum* vinegar was produced by natural microbial fermentation for duration of 42 days. According to Mokhtar et al. (2016), Rambutan vinegar contain the same amount of carbohydrate, protein and fat with the apple cider vinegar and Attap seed (nipa) vinegars. Rambutan vinegar has a moisture content of 81.47% whereas the value of ash content and carbohydrate content is 0%.

Previous study by Mokhtar et al. (2016) states that Rambutan vinegar has the highest value of protein content which is 0.27% when compared to other vinegars such as dokong vinegars, apple cider vinegars and nipa vinegar. The pH value of Rambutan vinegar is quite low which approximately around 3.48.

Besides, Rambutan vinegar contains 18.53% of total solid content. Total solids are measure of the amount of material dissolved in water. This material can include carbonate, bicarbonate, chloride, sulphate, phosphate, nitrate, calcium magnesium, sodium, organic ions and other ions (American Public Health Association, 1998).

2.4 pH Value

Gelatin is amphoteric which means it possesses acidic and alkali properties depending on the solution nature. Isoelectric point of gelatin is the pH at which the charge is neutral (Gelatin Manufacturers Institute of America [GMIA], 2012). The isoelectric point of gelatin ranges from 4.8 to 9.4. Alkali processed gelatins having lower isoelectric

points than acid processed gelatins. According to Stainsby (1987), gelatin can forms gels over a broad range of pH and variety of solutes. Type A gelatin has pH values ranging from 7 until 9 while Type B has smaller pH range of 4.7 to 5.4.

pH values of gelatin are variable and dependent on the type of acid used during pre-treatment process and extraction process especially during washing steps (Alfaro Alfaro, Balbinot, Weber, Tonial, & Machado, 2014). pH value affected the physical functionalities, gel strength viscosity, and melting point of gelatin. A higher pH value of gelatin may be due to efficient washing steps. pH is one of important parameters used to determine the quality of gelatin as it influenced other physiochemical properties such as the viscosity and gel strength. (Faradiella, Ningsih & Triastuti, 2017)

2.5 Colour Analysis

Gelatin's colour commonly ranges from pale yellow to dark amber. In fish gelatin, the colour is influenced by species of fish, raw material and extraction conditions. Dark colour of gelatin are usually caused by contaminant substances which are failed to be removed during extraction procedure (Alfaro et al., 2014). The lighter colour of gelatin may higher commercial satisfaction and preferable by the customers. However, the colour does not affects other functional properties of gelatin.

The colour of gelatin has a practical significance due to 60% of world gelatin production is applied by confectionery industry (GMIA, 2012). The lower the colour variation in the ingredients, the easier it would be to produce uniform products. In

addition to that, the lack of colour is associated with purity, hence, gelatin with paler colour is more desirable compared with gelatin of darker colour.

An efficient filtration system is required during the extraction process of gelatin in order to obtain gelatin with less impurities. For this study, Chroma meter is used to analyse and assess the surface colour of the gelatin. Data output is being analysed in terms of lightness (L^*), redness (a^*) and yellowness (b^*) values.

2.6 Texture Profile Analysis (TPA)

Texture profile analysis (TPA) is intended to simulate the action of tongue and teeth exerted on the gel of gelatin. TPA gives more information regarding physical characteristics of gelatin compared to gel strength and it measures textural properties of foods such as hardness, springiness, cohesiveness, gumminess and chewiness. It involves double compression test to determining the textural parameters. Hardness is associated to the gel structure's strength when it is under compression. It is the maximum force required to compress the sample, which is the peak force during the first compression cycle.

Cohesiveness is a measurement of the difficulty degree in breaking down the internal structure of the gel. In other words, it is the extent to which the sample could be deformed before rupture. Meanwhile, springiness or elasticity is the height that the sample recovers during the time that elapses between the end of the first compression and the start of the second. Chewiness is the work needed to chew a solid sample to a steady state of swallowing In previous studies, catfish skin gelatin showed a significantly high cohesiveness and gumminess compared to bovine gelatin using the concentration of 6.67

g / 100 g. From previous study by Mahmoodani, Sanaei, See, Yusop & Babji (2012), bovine gelatin had a springiness of 0.97 while the springiness of catfish skin gelatin is 0.99. Catfish skin and bovine gelatin had cohesiveness of 0.92 and 0.99, respectively.

Cohesiveness and springiness of catfish skins gelatin possess almost similar properties to bovine gelatin. However, catfish skins gelatin gave considerably lower value of hardness, chewiness and gumminess than bovine gelatin, when compared at the same concentrations. Previous studies showed that during the first compression by texture profile analyser, gel structure broken down into large pieces resulting in high springiness whereas, when gel broken down into small pieces it indicates low springiness (Mahmoodani et al, 2012). Characteristic of gelatin that give texture and stability to its finished product was the important attributes for high quality of gelatin.

2.7 Moisture Content

Moisture content is one of the most crucial analytical procedures that can be conducted. It is a quality factor that affects stability and preservation of products. Also, it can be a resistance towards food deterioration. Moisture content data are used to express results of another analytical determinations on a uniform basis. It can be defined as the percentage loss in weight of the gelatin sample. The dry matter that remains after the removal of moisture is known as total solids.

Previous studies by Alfaro et al. (2014) stated that commercial gelatin has a moisture content ranging between 9 to 14%. Gelatin can preserve a balance of 13% moisture and 46% at ambient relative humidity when it is maintained at room

temperature; 25°C. The moisture content is removed from the foodstuff by heating it at high temperature ranges 95-105°C inside drying oven.

2.8 Ash Content

Ash can be defined as an inorganic residue remaining after complete oxidation of organic matter in a foodstuff (Marshall, 2010). It indicates the total mineral content in food. There are two types of ashing namely dry ashing and wet ashing. Dry ashing is primarily used for proximate composition and for few types of specific mineral analyses. Meanwhile, wet ashing is used as a preparation for analysis of certain minerals. Low ash content contributes to a high gelatin quality where ash content should be lower than 0.5% (GMIA, 2012). Maximum ash content of 2.6% is usually accepted for food applications. Under the Food Act 1983 and Food Regulations 1985, ash content for gelatin powder cannot exceed 3%.

Appropriate demineralisation of fish skins should be accomplished in order to obtain gelatin with the lower ash content prior to gelatin extraction (Marshall, 2010). High temperature may cause the volatilization of certain elements especially potassium, sodium, chlorine and phosphorus. For dry ashing, the foodstuff is burn inside a muffle furnace for an appropriate temperature and time or until whitish grey ash is obtained. It is essential to determine of ash content as it could be used to analyse the nutritional value of catfish gelatin produced.

2.9 Protein Content

The protein content represents the maximum possible yield of gelatin expected from them (Muyonga et al., 2004). The protein content was determined by estimating the total nitrogen content using Kjeldahl method (Association of Official Analytical Chemists [AOAC], 2000). Kjedahl method involves of three steps starting from digestion, distillation and lastly, titration.

During digestion process, the foodstuff is digested in concentrated sulphuric acid with the aid of catalyst in order to convert the protein nitrogen to ammonia. After that, the step proceed with distillation process followed titration using hydrochloric acid until the solution turns to slight violet in colour.

The amount of hydrochloric acid used during the titration will be used during the calculation of the nitrogen content and crude protein content. Jongjareonrak et al. (2006) said that the raw material, chemical residuals after processing, or accidental mixing with other ingredients will influenced the protein, moisture, ash and fat contents.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Raw Materials

20kg of fresh African catfish were obtained from Agropark, Universiti Malaysia Kelantan, Jeli. The catfish were stored inside the chiller at Universiti Malaysia Kelantan's Food Laboratory with temperature ranges -20°C until further use.

3.1.2 Chemicals

The chemicals used in extraction of gelatin were acetic acid, Rambutan vinegar, sodium hydroxide, distilled water, paraffin, sulphuric acid, hydrochloric acid, Kjeldahl tablets, boric acid, ethanol, bromocresol green indicator and methyl red indicator.

3.1.3 Equipment

Equipment that utilized were beakers, water bath, thermometer, sieve, knife, chopping board, glass bottles, aluminium foils, Texture Analyzer, pH meter, Chroma meter, crucible, electric hot plate, muffle furnace, evaporating dish, drying oven, blender, Kjeldahl apparatus, measuring cylinder, cheesecloth, polyethylene bags, glass rod, petri dish, electronic balance, plastic tray, gloves, burette, conical flasks, retort stand and spatula.

3.2 Methods

3.2.1 Sample Preparation

The catfish skins were filleted and removed manually using a knife. Next, the catfish skins were cleaned thoroughly under running tap water for three times to remove attached flesh and were drained using cheesecloth. The cheesecloth was squeezed in order to remove excess water from the catfish skins. The washed skins were chopped into small pieces (2 to 3cm) and were packed in polyethylene bag. The skins were frozen at -20°C until further use (See et al., 2010).

3.2.2 Gelatin Extraction

The gelatin extraction were performed according to Gomez-Guillen and Montero (2001) with a little modification. 900g of catfish skins were thawed for overnight at 4°C and then washed with running tap water three times.

After that, the skins were soaked with 0.2 M sodium hydroxide (NaOH) at room temperature for 1 hour and were rinsed with tap water three times. The catfish skins were divided into six portion and were allowed to swell with addition of varying concentrations of acetic acid (CH_3COOH) and Rambutan vinegar which are 2%, 4% and 6% for 16 hours at room temperature.

Later, the samples were drained and rinsed three times under tap water before extracted with distilled water at 55 °C for 6 hours. The skin to water ratio were set to 1:8 (w/v). After that, the liquid portion was separated from the skins using a sieve. Then, the extracted solution was filtered again using cheesecloth.

The gelatin solutions were put into containers and oven dried at 45°C for 36 hours. Later, the dried gelatin films were grinded into powder using a blender. The gelatin samples were kept inside sealed polyethylene bag until further use.

3.2.3 Determination of Percentage Yield

According to Sarbon, Badii and Howell (2013), the extraction yield was expressed as ratio of weight of dried gelatin to the weight of cleaned fish skin using equation 1.0:

$$\text{Yield of gelatin (\%)} = \frac{\text{Weight of dried gelatin (g)}}{\text{Weight of cleaned skin (g)}} \text{ X } 100\% \quad (1.0)$$

3.2.4 Determination of pH Value

The pH was determined by following British Standard Institution [BSI] (1975) method. The 1% (w/v) gelatin solution was prepared by diluting catfish skin gelatin in distilled water at 60°C and was cooled at temperature at approximately 25°C. pH meter was used to determine the pH value. The pH reading was taken triplicate for each sample.

3.2.5 Determination of Colour Analysis

The 6.67% (w/v) of gelatin solution was prepared by mixing gelatin with distilled water at 65°C and poured into petri dish and stored in the chiller at 4°C for 18 hours for formation and maturation of gel (Gomez-Guillen & Montero, 2001). After that, the colour of the gelatin gel was determined by using the CR 400 Chroma meter and the values of *L**, *a** and *b** were measured. According to Widyasari & Rawdkuen (2014), *L**, *a** and *b** values indicates the lightness/brightness, redness/greenness and yellowness/blueness respectively.

3.2.6 Determination of Texture Profile Analysis

A 6.67% (w/v) gelatin solution was prepared following the British Standard Institution (1975). 7.5 g of the dry powder of catfish skins gelatin was mixed with 105 mL of distilled water. The mixture was left at room temperature for 30 minutes, allowing absorption of water and swelling of the gelatin Later, the mixture was heated at 65°C for 20 minutes allowing gelatin to be completely dissolved. The gelatin solution was poured into petri dish and was allowed to be cool at room temperature for 15 minutes. Then, the gelatin was kept in a chiller with temperature range 4°C for 18 hours.

The texture profile analysis (TPA) was conducted using CT3 Texture Analyzer prepared with 10 kg load cell. TA4/1000 probe with length of 20 mm and diameter of 38.1 mm was used with two cycle compression to compress the gelatin gel. The parameters used for setting up the texture analyser was shown in Table 3.1:

Table 3.1: Parameters for TPA settings

Parameter	Value
Pre-test speed	2.00 mm/s
Test speed	10.00 mm/s
Post-test speed	1.0 mm/s
Target mode	510 g
Trigger load	1 g

3.2.7 Determination of Moisture Content

An evaporating dish was washed in hot water and placed in a drying oven at 105°C for 1 hour. Next, the dish was cooled in desiccator until it reached room temperature. 5 g of gelatin was weighed (m_0) and put into the evaporating dish. The weight of the evaporating dish with the sample was recorded (m_1). The evaporating dish was placed in the drying oven at 105 ± 2 °C for 16 hours.

Then, the evaporating dish was placed and cooled in desiccator until it reached room temperature. The evaporating dish was weighed again (m_2). The moisture content was calculated based on equation 2.0:

$$\text{Moisture content (\%)} = \frac{m_1 - m_2\ (g)}{m_0\ (g)} \text{ X } 100\% \qquad (2.0)$$

3.2.8 Determination of Ash Content

The ash content was determined following the Gelatin Manufacturers Institute of America (2013) method. The crucible was ignited, cooled and weighed. 5 g of gelatin was weighed in the crucible and 2.0 g of paraffin was added to avoid loss due to swelling. The sample was heated using an electric hot plate until it was thoroughly charred.

Then, the sample was dried in a muffle furnace for 15 to 20 hours at 550 °C. After that, the sample was cooled in desiccator and weighed. The ash content was calculated using equation 3.0:

$$\text{Ash content (\%)} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \text{ X } 100\% \qquad (3.0)$$

3.2.8 Determination of Protein Content

The nitrogen content was measured using Kjeldahl method to determine the crude protein content of the gelatin. This method involves the process of digestion, followed by distillation process and lastly, titration process.

For digestion method, 1 g of gelatin sample was weighed and placed in a digestion tube. 1g of Kjeldahl powder containing Missouri catalyst and 12 mL of sulphuric acid were added into the tube. Then, heat was applied in fume cupboard to prevent excessive frothing. The digestion process was extended for 45 minutes. Next, the sample was left until it cooled completely and 80 mL of distilled water was added immediately. 50 mL of sodium hydroxide (NaOH) was added by using measuring cylinder to avoid ammonia lost.

For distillation method, the distillation apparatus was steamed up. A 30 mL solution made up of boric acid, distilled water, methyl red and bromocresol green was dispersed into receiving flask and placed on distillation system. The distillation tube of gelatin sample was submerged into the solution of the boric acid.

As for titration method, the alkaline ammonium borate formed was titrated with 0.1 M hydrochloric acid (HCl). The sample was titrated until the solution changes its colour from green to slightly violet. The volume of HCl used for titration was recorded (Patil, 2017; Reagents, 2015). The nitrogen content was calculated using equation 4.0:

$$\text{Nitrogen content (\%)} = \frac{(\text{ml of standard acid} - \text{ml of blank}) \times \text{N of acid} \times 1.4007}{\text{weight of sample (g)}} \quad (4.0)$$

Most proteins contain 16% of nitrogen content, therefore, the conversion factor is 6.25. Thus, the protein contein can be calculated using equation 5.0:

$$\text{Crude protein (\%)} = \text{Nitrogen content (\%)} \times 6.25 \quad (5.0)$$

3.2.10 Statistical Analysis

The statistical analysis was performed using SPSS software (SPSS Inc, Chicago, IL, USA). The data was analysed using one-way analysis of variance (ANOVA) and Duncan's multiple range test was performed in order to determine the significant differences between means.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Yield of Catfish Skin Gelatin

Table 4.1 indicates the percentage yield of the African catfish skin gelatin pre-treated using acetic acid and Rambutan vinegar of varying concentrations. Significant differences ($p \leq 0.05$) were observed among the gelatin yield in all the treatments. According to the results in Table 4.1, among acetic acid pre-treatments, 6% gave the highest yield of gelatin with 17.36%. Similarly, the highest yield from pre-treatments using Rambutan vinegar was obtained from concentration of 6% which is 16.37%.

Table 4.1: Percentage yield (mean±SD) of African catfish skin gelatin extracted using acetic acid and Rambutan vinegar of varying concentrations.

Concentration	Pre-treated with acetic acid	Pre-treated with Rambutan Vinegar
2%	14.12 ± 0.01^{e}	15.44 ± 0.01^{c}
4%	15.16 ± 0.01^{d}	10.54 ± 0.02^{f}
6%	17.36 ± 0.01^{a}	16.37 ± 0.01^{b}

Note: Different superscript letters in column represent significant different ($p \leq 0.05$) (n=3).

Figure 4.1 shows that gelatin yields in acetic acid pre-treatments increased as the concentration of acid increased from 2% to 6%. Similar trend was shown by pre-treatments using Rambutan vinegar with the exception from catfish skin pre-treated with 4% Rambutan vinegar where it gave a notably low yield value of 10.54% compared to other yields. Also, the gelatin yield from acetic acid pre-treatments were slightly higher than those pre-treated with Rambutan vinegar.

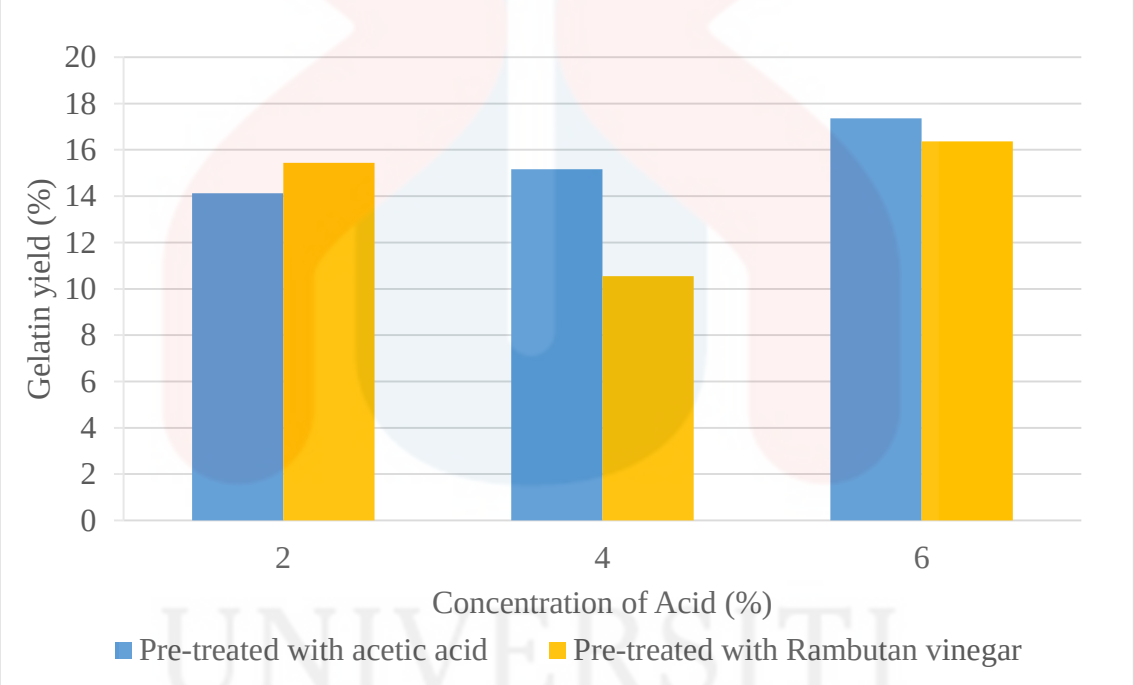

Figure 4.1: African catfish gelatin yield (%) pre-treated with acetic acid and Rambutan vinegar of varying concentrations.

The low yield of catfish skin gelatin from pre-treatment using 4% Rambutan vinegar was influenced by the condition during drying process. Previously, all the gelatins extracted were dried in drying oven at the temperature of 45°C until it is completely dried to obtain the dry form of gelatin (Rasli & Sarbon, 2015). Nevertheless, uneven drying occurred among the samples because many samples were placed in the oven

simultaneously. Thus, longer time taken for some of the gelatin samples to dry completely, which in this case, the gelatin pre-treated with 4% Rambutan vinegar. The gelatine yield and some quality properties of that gelatin were affected due to long drying period. High drying temperature caused protein degradation, thereby it produced protein fragments, thus, lowering the gelling ability (Sae-Leaw, Benjakul & Brien, 2015). In addition, some growth of mold on the gelatin also affects the yield of gelatine extracted from 4% Rambutan vinegar.

Overall, the gelatin yield from this study is lower compared to previous study made by Sanaei, Mahmoodani, See, Yusop, & Babji (2013) where the catfish gelatin yield was 21.79%. These differences were influenced by some aspects during extraction such as type and concentrations of acids used together with extraction methods (Zhou & Regenstein 2004). The type of acids used during pre-treatments affects the pH of the extraction solution which influenced the swelling and disintegration of covalent bonds in the collagen cross-links of the catfish skin and influenced the efficiency of extraction process (Gomez-Guillen & Montero, 2001).

Acetic acid pre-treatment provided a higher swelling power for acid labile cross-links destabilization at the telopeptide region and amide bonds of the triple helical structure of collagen as well as non-covalent intra- and inter-molecular bonds (Gomez-Guillen & Montero, 2001). Acetic acid has a higher ionic strength and lower pH than Rambutan vinegar. Therefore, even at the same concentration of acids, acetic acid facilitate the swelling process better and caused extra repulsive force among the collagen molecules (Gomez-Guillen & Montero, 2001) compared with Rambutan vinegar. Hence, with the loosen structure of swollen collagen, warm water could penetrate easily into the skin matrix (Stainsby, 1987) and obtaining higher yield of gelatin.

In addition to that, extraction methods also affects the yield of gelatin. According to Schrieber and Gareis (2007), when the alkali treatment is excessive, the collagen becomes soluble in cold water. During the washing steps, the collagen which dissolved in cold water also drained along with the water and resulted in low yield of gelatin. In addition to that, incomplete hydrolysis of collagen throughout the extraction process also affect the gelatin yield. (Jamilah & Harvinder, 2002).

As stated by Almeida & Lannes (2013), cotton that was used in filtration method also contributed to low gelatin yield since its absorbing power is high. Moreover, temperatures between 50 to 70°C is suitable for optimum gelatin extraction as lower temperature produced low yield whereas higher temperature might affect the gelatin quality (Tavakolipour,2011). The higher the yield of gelatin generated, the more efficient the treatment applied (Faradiella et al., 2017)

These results indicate that both the acid type and concentration have effects on the yield and gelatin extracts. It is proven that Rambutan vinegar can also be used as pre-treatment acid in gelatin extraction as the gelatin yield obtained were comparable to those gelatin pre-treated with acetic acid. Nevertheless, some modification and precaution needed to be done in order to improve the extraction hence, increase the yield. Drying process of gelatin solutions needed to be monitored regularly in order to avoid uneven drying and growth of mold.

4.2 pH analysis of Gelatin

According to Table 4.2, the pH values varied significantly ($p \leq 0.05$) from 4.67 to 5.71 depending on the acids and concentrations used in the gelatin pre-treatment. Gelatin extracted from African catfish skin after pre-treatment with 2% acetic acid had the highest pH value of 5.71.

pH values were significantly ($p \leq 0.005$) higher in gelatin pre-treated with Rambutan vinegar compared to those pre-treated with acetic acid. For both pre-treatments, it showed that as the concentrations of acid increased, the pH value of the extracted gelatin decreased and this is because higher acid concentrations has lower pH.

Table 4.2: pH values (mean±SD) of African catfish skin gelatin extracted using acetic acid and Rambutan vinegar of varying concentrations.

Concentration	Pre-treated with acetic acid	Pre-treated with Rambutan Vinegar
2%	4.91 ± 0.01^{c}	5.71 ± 0.01^{a}
4%	4.85 ± 0.01^{d}	5.69 ± 0.01^{a}
6%	4.67 ± 0.02^{e}	5.02 ± 0.02^{b}

Note: Different superscript letters in the same column represent the significant different ($p \leq 0.05$) (n=3)

Previous studies mentioned that Rambutan vinegar has acetic acid content ranging from 3.50-7.45% (Mokhtar et.al, 2016; Ninlanon & Puttame, 2010). According to Malaysian Food Regulation (1985) and United States Food and Drug Administration (USFDA), vinegar shall contain more than 4% w/v of acetic acid and shall not contain any mineral acid. In this study, the pH of Rambutan vinegar ranged between 3.41 and 3.44 whereas acetic acid has lower pH of 2.35 to 2.70.

The pH value of gelatin is significantly affected by the type and concentration of acids used during pre-treatments process as well as the handling during extraction process (Alfaro et al., 2014; Songchotikunpan et al., 2008). This is because the skins have been soaked with acid before undergoing washing before further processing so some of acid molecules might bound to skin, but only in a small amount. Therefore, acetic acid might integrate into the gelatin and caused the pH values of gelatin pre-treated with acetic vinegar lower than those gelatins pre-treated with Rambutan vinegar.

Pre-treatment using acetic acid and Rambutan vinegar had a different effect on catfish skins and resulted in a different final pH during gelatin extraction, leading to the difference in the yield and gel strength of the gelatin extracts. The swelling of catfish skins was greater in acetic acid compared to Rambutan as acetic acid has higher ionic strength. Nevertheless, Gomez-Guillen and Montero (2001) stated that if the pH conditions during the extraction were identical, even when different type or concentration of acid used in the pre-treatments, the final products will produce similar gelatin yields and gel strength. Higher yield of gelatin can be obtained from neutral and acid conditions. However, higher gel strength can only be attained from neutral or weak acid condition.

In some cases, despite of the total H concentration of each acid was the same, the ionized H+ available in solution was different, which it gave different effect on catfish

skins during pre-treatment process. Thus, a different final pH of solution during gelatin extraction led to the difference in the gelatin yield and the strength of gelatin gel.

The pH of the gelatin solution is also affected by the chemical treatment employed during the extraction (Gudmundsson & Hafsteinsson, 1997). The higher pH of the gelatin is possibly due to the efficiency of washings after chemical treatments before the extraction (Alfaro et al., 2014). In alkaline or acid extracting condition, some polypeptide chains of collagen were broken into small pieces. Combination of the two pre-treatments provided a proper pH for extraction, during which some cross-linkages could be further destroyed but with less breakage of polypeptide chain (Hinterwaldner 1977).Thus, process of neutralizing and washing the raw material before the extraction process is needed to be conducted perfectly so that contamination can be minimized.

According to GMIA (2012), the common specification for the pH of edible gelatin ranged from 3.8 to 5.5 for Type A gelatin whereas Type B gelatin ranged from 5 to 7.5 Therefore, gelatin extracted from this study were acceptable as edible gelatin as this result approached the standard set by GMIA and comparable to the pH of commercial gelatin ranged between 4-7.

Swelling capacity of collagen, extraction pH as well as ionic strength varies depends on the type of acid used and are crucial for the functional effectiveness of the extraction. Functional properties of gelatins such as gel strength, texture profile and melting point are dependent on pH (Gudmundsson & Hafsteinsson, 1997). A low pH can favour a maximum extraction rate but produces more degradation of lower-molecular weight peptides thus, effects the physical properties of gelatin.

4.3 Colour Analysis of Gelatin

Table 4.3 shows the colour values of the catfish skin gelatin extracted using acetic acid and Rambutan vinegar of various concentrations. There is significant difference ($p \leq 0.05$) between the lightness (L^*), redness (a^*) and yellowness (b^*) values of the gelatin extracted. In the cases of the Rambutan vinegar as pre-treatment acid, the L^* value were lower than gelatin pre-treated with acetic acid ($p \leq 0.05$). Nevertheless, there was an exception for gelatin pre-treated with 2% Rambutan vinegar as its L^* value is the highest (30.11) among all the gelatin extracted.

Table 4.3: Colour analysis (mean±SD) of African catfish skin gelatin extracted using acetic acid and Rambutan vinegar of varying concentrations.

Treatment	Concentration	Lightness (L^*)	Redness (a^*)	Yellowness (b^*)
Pre-treatment using acetic acid	**2%**	25.09 ± 0.40^{d}	3.30 ± 0.17^{e}	6.91 ± 0.10^{d}
	4%	29.35 ± 0.63^{a}	6.98 ± 0.70^{a}	10.42 ± 0.30^{b}
	6%	29.76 ± 0.36^{a}	5.88 ± 0.11^{b}	12.30 ± 0.08^{a}
Pre-treatment using Rambutan vinegar	**2%**	30.11 ± 0.41^{a}	4.56 ± 0.39^{cd}	7.62 ± 0.35^{c}
	4%	27.14 ± 0.53^{c}	4.00 ± 0.23^{d}	7.80 ± 0.19^{c}
	6%	28.41 ± 0.48^{b}	4.71 ± 0.30^{c}	10.96 ± 0.55^{b}

Note: Different superscript letters in the same column represent the significant different ($p \leq 0.05$) (n=3)

. The colour of gelatins were significantly affected by the types and concentrations of acid, raw materials used along with the extraction process (Gomez-Guillen, 2000). With acetic acid pre-treatment, pigments in the catfish skins were more likely removed to a greater extent to be compared with the skins pre-treated with Rambutan vinegar due to low pH causing higher acidity. The effect of the carryover of pigments from the catfish skins and led to a bit darker in the appearance of gelatin. Even though both acids were at the same concentration, the pH of acetic acid solution was lower than the pH of Rambutan vinegar. Hence, pH of pre-treatment acid significantly affects the colour of the gelatin. Previous study on Channel catfish observed that the pre-treatments at acidic solution resulted in transparent gelatin, whereas pre-treatments at basic solution resulted in darker colour of gelatin (Zhang et al., 2007)

The gelatins appears cloudy due to imperfect filtration process. In this study, the gelatin were filtered using sieve and cheesecloth. According to Muyonga et al., (2004) imperfect filtering affects the value of L^*, a^* and b^*. Gomez-Guillen (2000) states that the longer the soaking time of raw material in the acidic pre-treatment, the higher the value of L^*, whereas the lower the value of a^*, while the value of b^* remains stable. It is possible to obtain a light-coloured, dry collagen extract from catfish skins by solubilizing collagen with constant slow stirring overnight and removing the residual, not solubilized, dark skin. Also, the dark colour of the gelatin may be reduced by decreasing the extraction time, since long reaction times favours the Maillard reaction between protein and traces of carbohydrates in the raw material (Schrieber & Gareis, 2007).

The redness and yellowness values of the gelatin obtained utilizing the Rambutan vinegar pretreatment are lower than those gelatin obtained using acetic acid pretreatment, which is probably due to the occurrence of non-enzymatic browning reactions in the latter

cases. The darkening is caused by the Maillard reactions of free amino acids released by the acids in contact with C=O components in the gelatin (Mulyani et al., 2016; Jridi et al., 2015).

In general, the colour does not influence the functional properties and chemical quality of the gelatins (Jamilah & Harvinder, 2002). The lighter colour of gelatin gave more commercial satisfaction and being used to satisfy consumer preferences, hence, having only an aesthetic value. Generally, gelatin manufacture has a good process and equipment to clarify the impurities from the gelatin solution, such as chemical clarification and good filtration processes.

4.4 Texture Profile Analysis (TPA)

Table 4.4 summarises the texture profile analysis (TPA) of catfish skin gelatin pre-treated using acetic acid and Rambutan vinegar of varying concentrations. A significant ($p \leq 0.05$) differences were observed among the gelatin in terms of hardness, cohesiveness and chewiness. Meanwhile, there is no significant different between gelatin pre-treaments of 2% acetic acid and 2% Rambutan vinegar as well as between 6% of acetic acid and 6% Rambutan vinegar in term of gumminess and chewiness. In this study, the gelation of Catfish gelatin extracted using 4% Rambutan vinegar never occured.

Table 4.4: Texture profile analysis (mean±SD) of catfish skin gelatin extracted using acetic acid and Rambutan vinegar with varying concentrations.

Attributes	Pre-treated with acetic acid			Pre-treated with Rambutan Vinegar		
	2%	4%	6%	2%	4%	6%
Hardness (N)	629.33 ± 12.70^{b}	622.67 ± 10.97	700.33 ± 10.07^{a}	559.67 ± 10.26^{c}	-	553.33 ± 6.43^{c}
Cohesiveness	14.80 ± 0.20^{a}	12.24 ± 0.85	9.08 ± 0.08^{b}	1.00 ± 0.09^{c}	-	1.15 ± 0.04^{c}
Springiness (mm)	27.65 ± 0.02^{a}	17.70 ± 0.74	0.96 ± 0.31^{c}	27.04 ± 0.38^{a}	-	24.97 ± 1.08^{b}
Gumminess (N)	580.00 ± 8.66^{b}	721.67 ± 10.21	649.67 ± 4.51^{a}	581.67 ± 24.11^{b}	-	656.33 ± 14.19^{a}
Chewiness (N)	158.50 ± 8.55^{a}	187.60 ± 14.55	$127.23 \pm 10..87^{b}$	150.10 ± 9.68^{a}	-	143.37 ± 8.34^{b}

Note: Different superscript letters in the same row represent the significant different ($p \leq 0.05$) (n=3)
* - : Gel formation never occurred for this sample

Gel formation never occurred for pre-treatments using 4% Rambutan vinegar. Uneven drying occurred among the samples during drying process of gelatine extracted as many samples were placed in the oven simultaneously. Longer time taken for some of the gelatin samples to completely dry, which in this case, the gelatin pre-treated with 4% Rambutan vinegar. High drying temperature caused protein degradation, thereby it produced protein fragments, thus, lowering the gelling ability (Sae-Leaw, Benjakul & Brien, 2015). A weak gelatin gel was associated with the formation of small fragments. The gelatine yield and some quality properties of that gelatin were affected due to long drying period.

The hardness value of gelatin pre-treated with acetic acid were higher than those pre-treated with Rambutan vinegar. Gelatin pre-treated with 6% acetic acid had the highest hardness value of 700.33. Hardness for gelatin from acetic acid were significantly higher than those gelatin from Rambutan vinegar. Chandra & Shamsundar (2015) stated that the hardness is associated to the gel structure strength under compression. In other words, it is the maximum force needed to compress food between molar teeth. The hardness of gelatin was affected by several factors such as the composition of the protein or molecular weight distribution inside the gelatin, the extraction process, amino acid composition as well as the pH of the gelatin extracted (Karim & Bhat, 2008; Songchotikunpan et al., 2008; Gudmunsson & Hafsteinsson, 1997).

In this study, the cohesiveness of gelatin pre-treated with Rambutan vinegar were significantly ($p \leq 0.05$) lower than those pre-treated with acetic acid. Cohesiveness is a measurement of the degree of difficulty in breaking down the gel's internal structure. The cohesiveness indicates the ability of the food to hold together (Chandra & Shamsundar,

2015). Therefore, the higher the cohesiveness value, the higher the effort needed to break down the gelatin inside the mouth.

Springiness or elasticity is a measurement of how much the gel structure is broken down by the first compression. In this study, the springiness value of gelatin decreased as the concentration increased for both pre-treatment acids. High springiness values resulted from the gel structure broken into large pieces during the first compression (Lau, 2000). Higher value of springiness attribute indicates that it requires greater mastication energy in mouth.

Gumminess and chewiness of gelatin from Rambutan vinegar pre-treatments were comparable to gelatin from acetic acid pre-treatments. Gumminess is the product of hardness x cohesiveness. The gumminess values ranged from 580.00 to 721.67 whereas chewiness ranged from 127.23 to 187.60. Chewiness is defined as the product of gumminess x springiness. It is associated to the work needed to chew a solid food to a state ready for swallowing. Greater value of chewiness indicates the need to chew the food more times.

All the texture attributes in this study were higher than the previous study on catfish made by Sanaei et al., (2013) and commercial bovine gelatin. The differences in textural properties of gelatin gels at similar concentration could be explained by different amino acid concentrations, different molecular weight distributions and less degraded peptides of gelatins (Benjakul et al. 2009; Liu et al. 2009). Catfish skins gelatin is a good alternative for the production of soft gels in pharmaceutical industry.

However, in order to make a harder gel, the concentration of fish gelatin must be much higher than for bovine and porcine gelatin gels. Modification of the catfish skin gelatin extraction using chemical and enzymatic methods could improve the texture and

quality thus, may become the alternative to replace the use of mammalian gelatin in food and pharmaceutical industries.

4.5 Moisture content

The statistical analysis showed that there was a significant different among the moisture content of catfish skin gelatin extracted. The moisture content varies between 7.80% and 12.02%. The highest value of moisture content was obtained from 6% of acetic acid and the lowest was from 4% Rambutan vinegar. Moisture content from gelatin extracted with Rambutan vinegar were significantly lower than those gelatin extracted from acetic acid.

Table 4.5.: Moisture content (%) (mean±SD) of African catfish skin gelatin extracted using acetic acid and Rambutan vinegar of varying concentrations.

Concentration	Pre-treated with acetic acid	Pre-treated with Rambutan Vinegar
2%	8.23 ± 0.00^{e}	8.53 ± 0.11^{d}
4%	11.49 ± 0.08^{b}	7.80 ± 0.08^{f}
6%	12.02 ± 0.01^{a}	8.91 ± 0.00^{c}

Note: Different superscript letters in the same column represent the significant different ($p \leq 0.05$) (n=3)

Figure 4.2 shows that the moisture content increased as the concentration of acid increased in acetic acid pre-treatments. Similar trend was shown by pre-treatments using Rambutan vinegar with the exception from catfish skin pre-treated with 4% Rambutan vinegar where it gave a slightly low value of 7.80% compared to others.

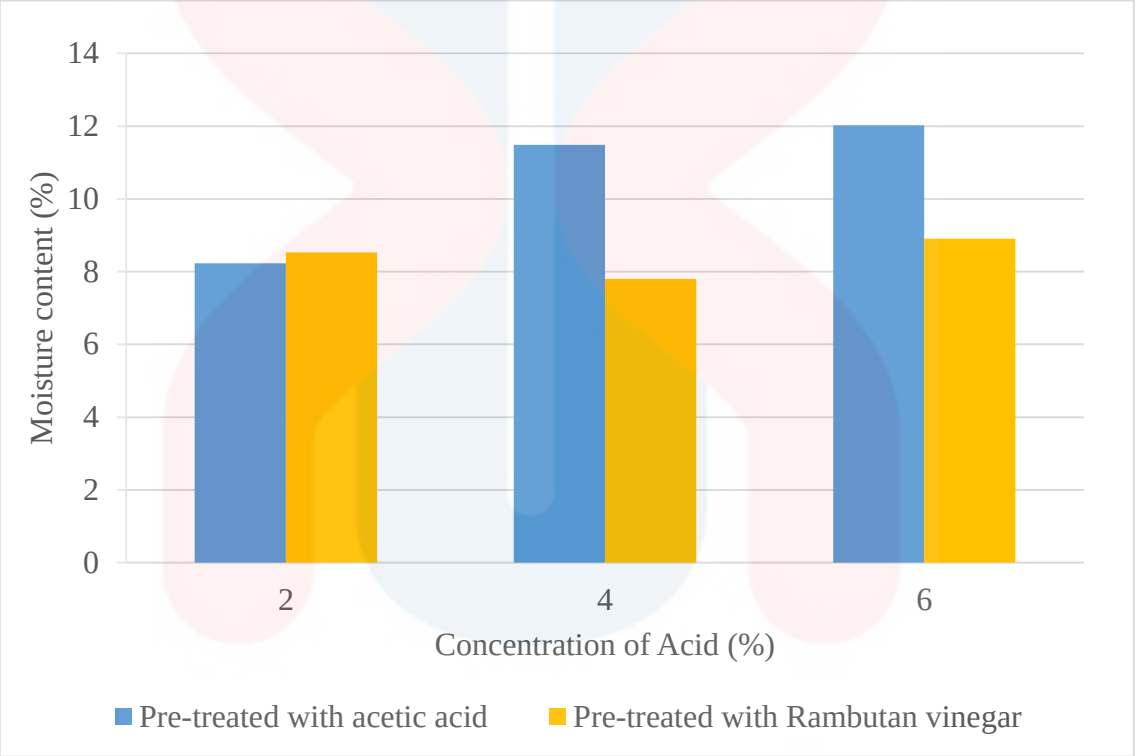

Figure 4.2: Moisture content (%) of catfish gelatin pre-treated with acetic acid and Rambutan vinegar of varying concentrations

The notably lower moisture value of gelatin pre-treated with 4% Rambutan vinegar compared to 2% and 6% of Rambutan vinegar was due to extra loss of moisture from long drying period in the oven. The moisture content in this study were lower than the standard set by GMIA which must not exceed 16%. Also, the moisture content in lower than commercial gelatin which is 12.21% (GMIA, 2012).

The moisture content of the catfish skin gelatin in this study were lower than the standard set and is suitable as an edible gelatin. Also, under the Food Act (2011) Malaysia, fish skin gelatin in the study conforms to the regulations because it contains less than 16% moisture.

Low moisture content increases the shelf life of gelatin as well as influences the rheological properties such as elasticity and viscosity of the products. Moisture content influenced the gelatin's shelf life as it affects the metabolic activity during the storage of gelatin. Metabolic activity such as microbes and enzymes activities might cause some changes in properties and nutritional value of gelatin.

Schrieber and Gareis (2007) say that the ideal humidity is 8–12% and a moisture content above 16% is not desirable because it presents a risk of lumping and microbiological growth and will damage gelatin by causing it to be sticky. Meanwhile, very low values of moisture (6–8%) lead to a very hygroscopic gelatin.

The higher moisture content in acetic acid may probably influenced by contribution of chemical residuals after treatments or interference with other substances. Gudmundsson & Hafsteinsson (1997) stated that the moisture content varies not only with the extent of drying, but also with the humidity during storage and the permeability to moisture of the packaging material.

4.6 Ash content

A significant different was observed among the ash content of gelatin from all treatments. The ash content of gelatin with acetic acid pre-treatments varied between 1.50% and 1.74%. Meanwhile, gelatin obtained from Rambutan vinegar pre-treatments varied from 0.77% to 0.82%. The lowest ash content was from gelatin extracted with 2% Rambutan vinegar. Overall, ash content from gelatin extracted with Rambutan vinegar was significantly ($p \leq 0.05$) lower than gelatin with acetic acid pre-treatments.

Table 4.6: Ash content (mean±SD) of African catfish skin gelatin extracted using acetic acid and Rambutan vinegar of varying concentrations.

Concentration	Pre-treated with acetic acid	Pre-treated with Rambutan Vinegar
2%	1.50 ± 0.00^{c}	0.77 ± 0.00^{f}
4%	1.55 ± 0.01^{b}	0.78 ± 0.00^{e}
6%	1.74 ± 0.01^{a}	0.82 ± 0.01^{d}

Note: Different superscript letters in the same column represent the significant different ($p \leq 0.05$) (n=3)

Ash content in the catfish skin was in the range of 0.77-0.82% which did not reached the maximum limit of 2.6% and should not exceed 2% for the edible gelatin according to the regulation by GMIA. In order to make edible gelatin, the ash content should be improved prior to product development.

The ash content of all the gelatins obtained was comparatively low. This was probably due to the ionization process that reduced the mineral content. From this analysis, it can be concluded that the concentration of acid increased, the ash content also increased. This is because higher concentration of acid indicates higher acidity thus the acid molecules might incorporated into the gelatin pre-treated with acetic acid and contributed to the higher ash content in the resulting gelatin.

Benjakul et al. (2009) stated that high quality of gelatin should contain no more than 0.5% ash. Moreover, Marshall (2010) stated lower ash content contributes to a high quality of gelatin and gelatin with a maximum ash content of 2.6 % is normally accepted for food applications. The ash content of catfish skin gelatin in this study was lower than the recommended maximum of 2.6 %. Under the Food Act (2011) Malaysia, the Food Act 1983 and Food Regulations 1985, the ash content for gelatin powder should not exceed 3%, the low ash content showed that gelatins extraction were done effectively.

Ash content reported to vary with the type of the raw materials, the extraction method and the mineral content in the raw materials. This indicates that the extraction process needs special attention to result in lower ash content toward minimum level. An appropriate demineralisation of the fish skins should be accomplished prior to gelatin extraction to obtain the gelatin with the lower ash content (Karim & Bhat, 2009).

The manufacture of fish bone gelatin may require improvement of the leaching process by adding an ion exchange step to remove the salts through application of a counter-current process. The high content of ash is due to improper washing method during extraction. Ion exchange method should be used to remove excessive minerals (Muyonga et al. 2004).

4.7 Protein content

Statistical analysis indicated that both Rambutan vinegar and acetic acid pre-treatments had highly significant effect (P≤0.05) on protein content of catfish skin gelatin. Protein content from catfish gelatin pre-treated using Rambutan vinegar ranged 89.70% to 95.77%. Meanwhile, the gelatin under acetic acid pre-treatments had a protein content ranged 85.36% to 92.24%. The protein content of gelatin pre-treated with Rambutan vinegar were significantly higher than those pre-treated with acetic acid. The values were comparable with the protein content of commercial gelatin which is 89. 63%.

Table 4.7: Protein content (mean±SD) of African catfish gelatin extracted using acetic acid and Rambutan vinegar of varying concentrations.

Concentration	Pre-treated with acetic acid	Pre-treated with Rambutan Vinegar
2%	92.24 ± 1.24^{b}	95.77 ± 0.32^{a}
4%	89.44 ± 1.42^{c}	94.17 ± 1.02^{a}
6%	85.36 ± 0.44^{d}	89.70 ± 0.48^{c}

Note: Different superscript letters in the same column represent the significant different (p≤0.05) (n=3)

The analysis showed that protein content of gelatin from catfish skin had decreased with increasing of concentration of Rambutan vinegar and acetic acid. This is because high acid concentrations increased the tendency of the acids to hydrolyze the

peptide bond. Therefore, there will be a loss of protein in catfish skin during the pre-treatment process.

According to Karim and Bhat (2009), the concentration of acetic acid solution resulted in the termination of high hydrogen bonds and the opening of the coil structure of collagen in excess so that some amino acids extracted and separated from the collagen and carried away by water washing, so that the protein content obtained is low.

Crude protein of gelatin can be used to evaluate the purity of the gelatin. The catfish skin gelatin obtained in this study had met the commercial requirement of the protein content. The protein content represents the maximum possible yield of gelatin that could be extracted from the skin but it is not a direct indication (Muyonga et al. 2004).

Nevertheless, the composition of hydroxyproline (hyp) in the collagenous material has been suggested as a better indicator to determine the yield of gelatin extracted from fish skin (Nalinanon, Benjakul, Visessanguan, & Kishimura, 2008). Jongjareonrak et al. (2006) suggest that the high protein content and the less moisture, ash and fat contents are determined by raw material or may be contributed by the residual of chemicals after processing, or also the possibility of mixing with other ingredients.

CHAPTER 5

CONCLUSION

5.1 Conclusion

In conclusion, the highest gelatin yield of was obtained from gelatin pre-treated with 2% acetic acid which is 17.36%. The type of acids and concentrations influenced the yield of the gelatin. Other factors such as handling technique during extraction methods and effect from oven drying may also affect the yield percentage. pH of gelatin extracted from both pre-treatments varied from 4.67 to 5.71. The gelatin pre-treated with Rambutan vinegar gave a significantly higher pH value compared to those pre-treated with acetic acid. The colour of gelatin is significantly different in terms of lightness, redness and yellowness for both pre-treatments. Some improvements need to be done in order to increase the lightness of the gelatin. For texture profile analysis, the texture attributes such as hardness, cohesiveness springiness, gumminess and chewiness obtained high values.

Proximate analysis of gelatin pre-treated with Rambutan vinegar gave positive results where the moisture (7.80-12.02%), ash (0.77-1.74%) and protein content (85.36-95.77%) of all treatments followed the standard set by Malaysian Food Act (2011).

From the study, the results represented high value in protein content, but low value for moisture and ash content in gelatin pre-treated with Rambutan vinegar compared with gelatin pre-treated with acetic acid. The optimum concentration of Rambutan vinegar that is suitable for gelatin acid pre-treatments was 6% as it obtained the highest yield and better physicochemical properties compared with concentrations of 2% and 4%.

Therefore, it can be concluded that gelatin from Rambutan vinegar can be an alternative to replace acetic acid as gelatin pre-treatments acid. Nevertheless, some modifications need to be done in order to increase the yield and improve the physicochemical properties such as colour and texture of the gelatin.

5.2 Recommendations

It was proven that Rambutan vinegar may become an alternative of acid used for gelatin pre-treatments. Further study should be conducted to evaluate other properties of gelatin such as gel strength, viscosity, gelling and melting temperatures as well as amino acid composition. This is important in order to evaluate whether the properties of gelatin that undergo Rambutan vinegar pre-treatment are comparable with those commercial gelatin. The extraction process needed to be optimized in order to gain higher yield of gelatin (Karim & Bhat, 2009). This is because the washing procedure and filtration method used influenced the gelatine yield. Besides, the usage of enzymes and proper filtration process during the extraction procedure should be considered in order to improve other rheological properties mainly regarding the colour and texture of gelatin.

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APPENDIX A

Table A.1: Statistical Analysis of One Way (ANOVA) for the yield percentage of gelatin, pH value, colour, as well as moisture, ash and protein content of African catfish skin gelatin pre-treated using acetic acid and Rambutan vinegar of varying concentrations.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Gelatin Yield	Between Groups	84.399	5	16.880	217026.629	.000
	Within Groups	.001	12	.000		
	Total	84.400	17			
pH of Gelatin	Between Groups	3.017	5	.603	3878.607	.000
	Within Groups	.002	12	.000		
	Total	3.019	17			
Moisture	Between Groups	48.243	5	9.649	2470.493	.000
	Within Groups	.047	12	.004		
	Total	48.290	17			
Ash	Between Groups	3.043	5	.609	21911.520	.000
	Within Groups	.000	12	.000		
	Total	3.044	17			
Protein	Between Groups	210.733	5	42.147	49.580	.000
	Within Groups	10.201	12	.850		
	Total	220.934	17			
Lightness	Between Groups	54.404	5	10.881	47.544	.000
	Within Groups	2.746	12	.229		
	Total	57.150	17			
Redness	Between Groups	26.461	5	5.292	38.737	.000
	Within Groups	1.639	12	.137		
	Total	28.101	17			
Yellowness	Between Groups	71.482	5	14.296	149.457	.000
	Within Groups	1.148	12	.096		
	Total	72.630	17			

Table A.2: Statistical Analysis of One Way (ANOVA) for the texture profile analysis of African catfish skin gelatin pre-treated using concentration of 2% and 6% acetic acid and Rambutan vinegar.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Hardness	Between Groups	42830.000	3	14276.667	139.511	.000
	Within Groups	818.667	8	102.333		
	Total	43648.667	11			
Cohesive	Between Groups	403.415	3	134.472	9628.036	.000
	Within Groups	.112	8	.014		
	Total	403.526	11			
Springiness	Between Groups	1485.673	3	495.224	1406.921	.000
	Within Groups	2.816	8	.352		
	Total	1488.489	11			
Gumminess	Between Groups	15694.917	3	5231.639	23.834	.000
	Within Groups	1756.000	8	219.500		
	Total	17450.917	11			
Chewiness	Between Groups	1579.267	3	526.422	5.939	.020
	Within Groups	709.053	8	88.632		
	Total	2288.320	11			

Table A.3 Post Hoc Test of Yield of Gelatin

Duncan[a]

Treatment	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
4% Rambutan Vinegar	3	10.5400					
2% Acetic Acid	3		14.1167				
4% Acetic Acid	3			15.1567			
2% Rambutan Vinegar	3				15.4367		
6% Rambutan Vinegar	3					16.3667	
6% Acetic Acid	3						17.3567
Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.4 Post Hoc Test of pH of Gelatin

Duncan[a]

Treatment	N	Subset for alpha = 0.05				
		1	2	3	4	5
6% Acetic Acid	3	4.6733				
4% Acetic Acid	3		4.8500			
2% Acetic Acid	3			4.9067		
6% Rambutan Vinegar	3				5.0200	
4% Rambutan Vinegar	3					5.6933
2% Rambutan Vinegar	3					5.7133
Sig.		1.000	1.000	1.000	1.000	.073

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.5 Post Hoc Test of Moisture Content of Gelatin

Duncan[a]

Treatment	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
4% Rambutan Vinegar	3	7.8033					
2% Acetic Acid	3		8.2300				
2% Rambutan Vinegar	3			8.5333			
6% Rambutan Vinegar	3				8.9100		
4% Acetic Acid	3					11.4933	
6% Acetic Acid	3						12.0167
Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.6 Post Hoc Test of Ash Content of Gelatin

Duncan[a]

Treatment	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
2% Rambutan Vinegar	3	.7700					
4% Rambutan Vinegar	3		.7800				
6% Rambutan Vinegar	3			.8167			
2% Acetic Acid	3				1.5000		
4% Acetic Acid	3					1.5533	
6% Acetic Acid	3						1.7400
Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.7 Post Hoc Test of Protein Content of Gelatin

Duncan[a]

Treatment	N	Subset for alpha = 0.05			
		1	2	3	4
6% Acetic Acid	3	85.3567			
4% Acetic Acid	3		89.4433		
6% Rambutan Vinegar	3		89.7033		
2% Acetic Acid	3			92.2400	
4% Rambutan Vinegar	3				94.1700
2% Rambutan Vinegar	3				95.7733
Sig.		1.000	.736	1.000	.055

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.8 Post Hoc Test of Lightness of Gelatin

Duncan[a]

Treatment	N	Subset for alpha = 0.05			
		1	2	3	4
2% Acetic Acid	3	25.0933			
4% Rambutan Vinegar	3		27.1433		
6% Rambutan Vinegar	3			28.4067	
4% Acetic Acid	3				29.3467
6% Acetic Acid	3				29.7600
2% Rambutan Vinegar	3				30.1100
Sig.		1.000	1.000	1.000	.087

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.9 Post Hoc Test of Redness of Gelatin

Duncan[a]

Treatment	N	Subset for alpha = 0.05				
		1	2	3	4	5
2% Acetic Acid	3	3.3000				
4% Rambutan Vinegar	3		3.9967			
2% Rambutan Vinegar	3		4.5600	4.5600		
6% Rambutan Vinegar	3			4.7133		
6% Acetic Acid	3				5.8767	
4% Acetic Acid	3					6.9833
Sig.		1.000	.087	.621	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.10 Post Hoc Test of Yellowness of Gelatin

Duncan[a]

Treatment	N	Subset for alpha = 0.05			
		1	2	3	4
2% Acetic Acid	3	6.9067			
2% Rambutan Vinegar	3		7.6167		
4% Rambutan Vinegar	3		7.8000		
4% Acetic Acid	3			10.4167	
6% Rambutan Vinegar	3			10.9600	
6% Acetic Acid	3				12.3033
Sig.		1.000	.482	.052	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.11 Post Hoc Test of Hardness of Gelatin

Duncan[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
6% Rambutan Vinegar	3	553.3333		
2% Rambutan Vinegar	3	559.6667		
2% Acetic Acid	3		629.3333	
6% Acetic Acid	3			700.3333
Sig.		.465	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.12 Post Hoc Test of Cohesiveness of Gelatin

Duncan[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
2% Rambutan Vinegar	3	1.0000		
6% Rambutan Vinegar	3	1.1467		
6% Acetic Acid	3		9.0833	
2% Acetic Acid	3			14.8000
Sig.		.167	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.13 Post Hoc Test of Springiness of Gelatin

Duncan[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
6% Acetic Acid	3	.9600		
6% Rambutan Vinegar	3		24.9667	
2% Rambutan Vinegar	3			27.0433
2% Acetic Acid	3			27.6500
Sig.		1.000	1.000	.246

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.14 Post Hoc Test of Gumminess of Gelatin

Duncan[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
2% Acetic Acid	3	580.0000	
2% Rambutan Vinegar	3	581.6667	
6% Acetic Acid	3		649.6667
6% Rambutan Vinegar	3		656.3333
Sig.		.894	.597

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.15 Post Hoc Test of Chewiness of Gelatin

Duncan[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
6% Acetic Acid	3	127.2333	
6% Rambutan Vinegar	3	143.3667	143.3667
2% Rambutan Vinegar	3		150.1000
2% Acetic Acid	3		158.5000
Sig.		.069	.096

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

APPENDIX B

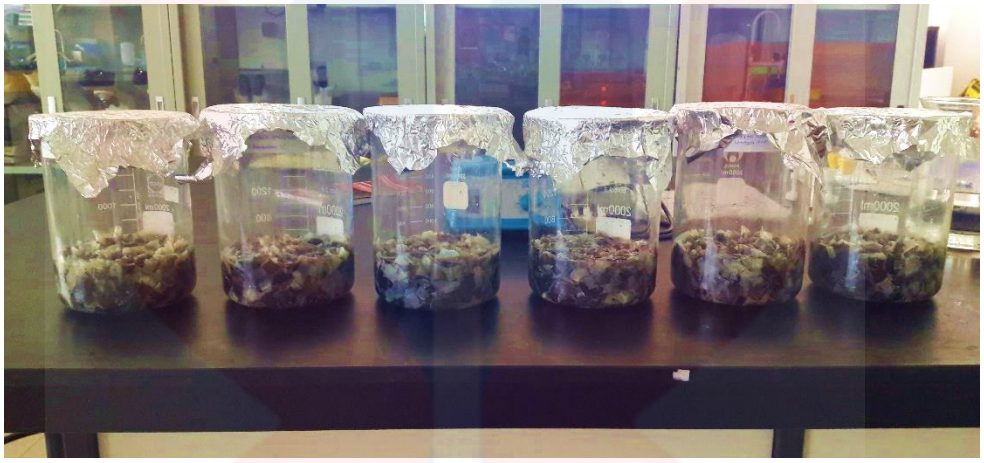

Figure B.1: The alkaline pre-treatments on African catfish skins

Figure B.2: The swelling condition of African catfish skin after being pre-treated with 2% acetic acid and 2% Rambutan vinegar

Figure B.3: The drying process of gelatin solution inside drying oven at 45°C until the samples were dried completely.

Figure B.4: TPA of African catfish gelatin gel using CT3 Texture Analyser

Figure B.5: Moisture analysis of African catfish gelatin

Figure B.6: The gelatin samples were put inside muffle furnace for the analysis of ash content